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Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products

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Abstract

The phenolic compounds in maple sap and syrup were extracted at different periods of the season and were separated to collect the glycosylated compounds and the aglycone compounds. The antioxidant and antiradical activities of each phenolic compound were studied using the thiobarbituric acid reactive substances (TBARS) assay and the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) decoloration test to measure the free radical scavenging. The results showed that in general the phenolic compounds had a good antioxidant and antiradical properties. The glycosylated compounds from maple sap and maple syrup showed a better activity than the aglycones. The antimutagenic effects of each phenolic compounds from maple sap and syrup were also investigated as the inhibition of SOS induction by chemical agents in *Salmonella typhimurium* TA1535/pSK1002 containing the fusion gene umuC-lacZ. Induction of the SOS gene (*umu*C) expression was assayed by measuring accumulated β-galactosidase activity using a modified Umu test. The antimutagenic properties were studied per se and after metabolisation by S9 fraction. The results showed that an optimum of antimutagenic properties of the glycosylated metabolites phenolic compounds from sap and syrup was observed at 75% of the season for the sap and at 25% of the season for the syrup. A higher antimutagenic activity was observed at 25% and 100% of the season for aglycones present in syrup and at 75% of the season for aglycones present in sap.
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1. Introduction

Oxidative stress by free radicals is an important event in the cell that can cause aging and human degenerative diseases including, cancer, heart diseases, multiple sclerosis, Parkinson's disease, autoimmune disease and senile dementia. Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds as a consequence of chemical or biological aggression may cause peroxidation of polyunsaturated fatty acids of cell membranes and liberation of toxic substances such as free radicals. Studies concerning the relationship between the morbidity due to cancer and heart diseases and the consumption of fruits and vegetables indicated that polyphenols present in large amount in fruits and vegetables have a significant impact on the morbidity decrease from these diseases (Heim, Tagliaferro, & Bobilya, 2002; Hertog, Hollman, & Van de Putte, 1993; Rice-Evans, 2001). Attention has hence been focused in recent years on antioxidant products from natural sources isolated from plant products. Polyphenolic compounds are found mainly in fruits and vegetables as secondary plant metabolites. Many polyphenols such as kaempferol, quercetin, luteolin,

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myricetin and catechin express strong antioxidative, antiiflamatory, antiallergic and antineoplasic properties (Balasinska & Troszynska, 1998). The high antioxidant activity of plant phenolic compounds attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals, playing a role in preventing many diseases.

The discovery and exploration of chemical compounds with antimutagenic and anticarcinogenic potency is at the present time of great importance because of the undesirable consequences of an increased rate of mutations and the related possible risks of cancer in humans (Kaur & Saini, 2000). Human epidemiology has indicated that cancer risk may be modified by changes in dietary habits or dietary components. Humans ingest large numbers of naturally occurring antimutagens and anticarcinogens, like the phytochemicals, in our food. These antimutagens and anticarcinogens may inhibit one or more stages of the carcinogenic process and prevent or delay the formation of cancer. Thus, studies on antimutagens in food are important to research on the physiological functionality of food components.

Maple syrup is one of the most important plant products in Québec, Canada, and represents 72% of the world production (Dumont, Saucier, Allard, & Arouze, 1993). Maple syrup is the product resulting from thermal processing of the sap from *Acer saccharum*. Maple sap represents a solution in which sucrose is the major component; however other compounds like organic acids, minerals and phenolic compounds have been reported (Kermasha, Goetghebeur, & Dumont, 1995; Kuentz, Simard, Zee, & Desmarais, 1976; Mollica & Morselli, 1984). Phenolic compounds, widely distributed in plants, contribute to the sensory properties associated with food quality such as color, aroma and may have potential health benefits, including reduction of cancer risk (Macheix, Fleuriet, & Billot, 1990). The analysis of phenolic compounds from maple sap and syrup have been conducted and from the many reported studies (Côté, 2003; Deslauriers, 2000) none had investigated the antioxidant, antiradical and antimutagenic activities of these compounds.

The aim of the present study was to evaluate the antioxidant, antiradical and antimutagenic activities of total phenolic compounds and phenolic compound extracts from maple sap and syrup collected at different periods of the season.

2. Materials and methods

2.1. Samples

Maple sap and syrup samples were provided by the Centre de recherche, de développement et de transfert technologique en acériculture (ACER, St-Hyacinthe, Québec, Canada). The samples were collected at different periods of the season 2002; 0%, 25%, 50%, 75% and 100% of the season (0% being the beginning and 100% the end of the season).

2.2. Extraction of phenolic compounds

Extraction of phenolic compounds from maple sap and syrup was achieved according to slight modifications of Kermasha et al. (1995) method. The sap and syrup samples (500 ml) were adjusted to pH 7. Three successive extractions in ethyl acetate (Fisher Scientific, Nepean, ON, Canada) were done. The first extraction was done using 500 ml of ethyl acetate and for the two last ones; a volume of 250 ml was used. A 2 l separating funnel was used for the extraction. During each extraction period, 10 min of agitation were used in order to separate the organic phase from the aqueous phase. The organic phase containing the phenolic compounds was recuperated after each extraction and kept at 4 °C. The three organic phases were pooled and mixed with 100 ml of deionised water in order to eliminate the presence of residual sugars. Anhydrous Na_2SO_4 was used to dry the organic phase and then it was filtered on Whatman no. 1 filter (Fisher Scientific). Complete evaporation of ethyl acetate extract was done using the SpeedVac Automatic evaporation system (Savant System, Holbrook, NY). The dry extract was dissolved in methanol (HPLC grade, Fisher Scientific) and dried under nitrogen in preweighed vials.

2.3. Quantification of phenolic compounds

The HPLC analyses were performed on a ProStar 230 (Varian Canada Inc., Mississauga, ON, Canada), equipped with a ternary pump delivery system, a Rheodyne injection valve (500 µl capacity, Waters Ltd., Dorval, QC, Canada) and a ProStar 330 diode-array UV–Vis detector (Varian); integration and data elaboration were performed using Star Chromatography Workstation software (Varian). A Varian analytic column C_{18}, 5 µm, 7.8 × 300 mm column was used. All solvents were filtered with 0.45 µm Millipore (Millipore Canada Ltd., Etobicoke, ON, Canada) filter disk and degassed with helium. A gradient elution was carried out using the following solvent systems: mobile phase A, double distilled water/acetonitrile (Laboratoires Mat, Beauport, QC, Canada)/formic acid (Fluka, Oakville, ON, Canada), (94/5/1, v/v/v); mobile phase B, double distilled water/acetonitrile/formic acid (69./30/1, v/v/v). The linear gradient elution system was: 100–90% A from 1 to 15 min and 90% A to 100% B from 15 to 50 min, keeping 100% B for 10 min, returning to 100% A followed by equilibration for 10 min before injection. Twenty µl of total phenolic compounds dissolved in methanol were injected after filtration through a

0.45 μm filter disk. The flow rate was 0.7 ml min^{-1} and the detection was performed at 280 nm. Total phenolic compounds concentration expressed as gallic acid equivalent was determined with a standard curve ($r^2 = 0.991$) made by concentrations from 0.0050 to 0.0175% (w/v) of gallic acid solution dissolved in 10% (v/v) methanol. This total phenolics concentration was determined using the summed peak area of the different phenolic extracts, and results were expressed as *g* of gallic acid equivalent (GAE) per 100 g of extract. Chromatograms obtained from maple sap and maple syrup during the season are presented Fig. 1. Some peaks were identified by comparison with standards and the retention times of known maple phenolic compound peaks. The phenolic compound standards were obtained from Sigma–Aldrich (Oakville, ON, Canada).

2.4. Separation of the glycosylated and aglycone compounds

The separation of the glycosylated and aglycone compounds from the maple extracts was conducted by dissolving 5 mg of total phenolic extracts from maple sap and syrup in 1 ml of methanol (Fisher Scientific, Nepean, ON, Canada) in order to apply the sample to a Amberlite XAD-2 resin (Supelco, Oakville, ON, Canada) conditionned in SR 10/50 column (Amersham Biosciences Corp., Baie d'Urfé, QC, Canada) (300 mm × 100 mm i.d.) with methanol 100% (grade HPLC, Fisher Scientific). The glycosylated extract was eluted with a methanol/water (60/40, v/v) solution (198 ml) using a peristaltic pump at flow of 0.5 ml/min. The glycosylated fraction was collected in

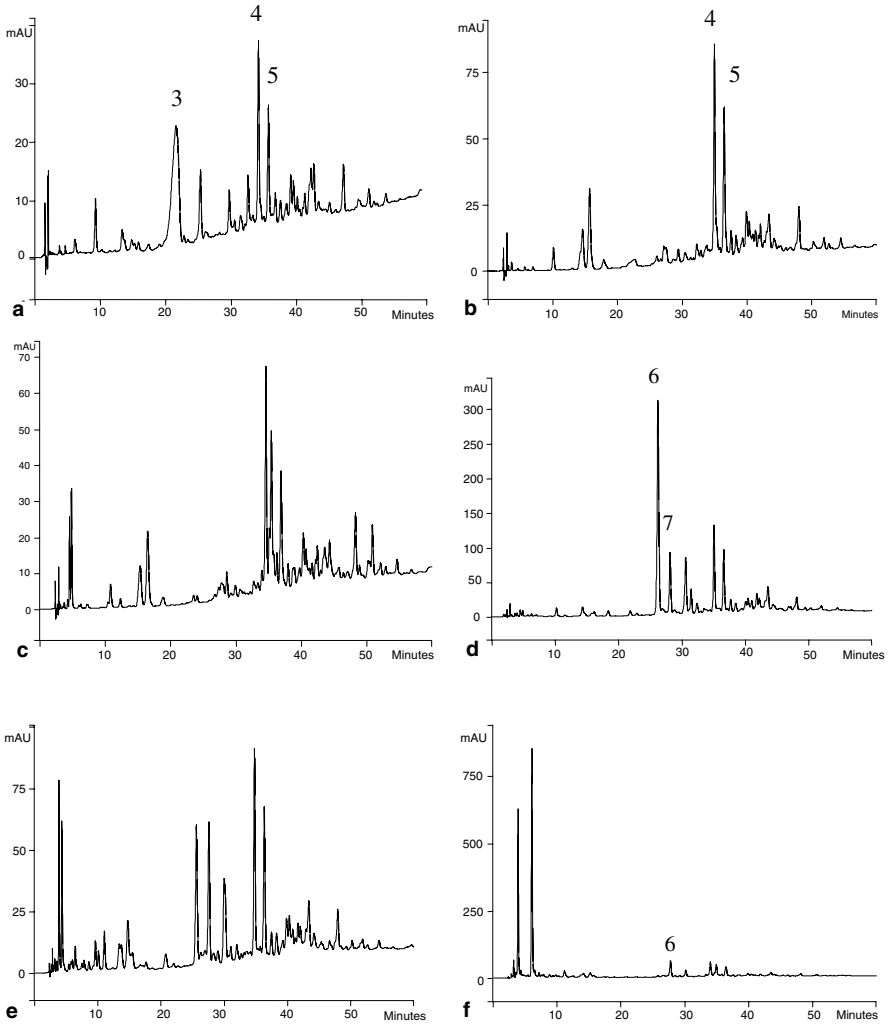

Fig. 1. Chromatograms of Phenolic compounds obtained from maple sap and maple syrup during the season: (a) sap, beginning of the season; (b) sap, mid-season; (c) sap, end of the season; (d) syrup, beginning of the season; (e) syrup, mid-season and (f) syrup, end of the season. (Peak 3) Coniferol; (Peak 4) syringaldehyde; (Peak 5) hydroxycinnamic acid; (Peak 6) Flavonol; (Peak 7) hydrobenzoic acid (products of degradation of phenolic compounds).

a 250 ml amber bottle. The solvent was removed from the fraction using the SpeedVac Automatic evaporation system (System Savant, Holbrook, NY). The sample was dissolved in 2 ml of methanol (Fisher Scientific) and dried under nitrogen in a preweighed vial and stored at −20 °C until analyzed. After the collection of the glycosylated fraction, the aglycone fraction was eluted in the XAD-2 column. This was done using 289 ml of a methanol/acetonitrile (Fisher Scientific) (50/50, v/v) solution. The first 7 ml were used to condition the column. The following 282 ml recuperated in a 300 ml bottle were evaporated with the SpeedVac. The sample was dissolved in 2 ml of methanol and dried under nitrogen in a preweighed vial and stored at −20 °C until analyzed.

2.5. Determination of antioxidant activity (AA)

The determination of the antioxidant activity of phenolic compounds from maple sap and syrup collected at different period of the season was done using a microtechnique based on the non-enzymatic peroxidation of rat liver microsomes method (Esterbauer, Cheeseman, Dianzani, Poli, & Slater, 1982) modified by Lessard (1995) where artificial membranes were used instead of rat liver microsomes, in order to obtain a more stable and reproducible system. This test measures by spectrophotometry the TBARS (thiobarbituric reactive substances) concentration producted during the peroxidation of liposomes exposed to iron ions in 20 mM phosphate buffer solution in presence of ascorbate. The antioxidant activity is equivalent to the lipid peroxidation inhibition capacity.

2.5.1. Liposomes preparation

Liposomes were formed by an injection method, as described by Batzri and Korn (1973). Linoleic acid (Sigma-Aldrich, Oakville, ON, Canada) was dissolved in 95% ethanol. The mixture was injected into phosphate buffer (20 mM, pH 7.4) in a proportion of 1:9 (v/v), using an hypodermic syringe fitted with a fine needle (G26).

2.5.2. Control solution preparation

Positive controls were ascorbic acid (vitamin C) (Laboratoires Mat, QC, Canada) for the hydrophilic compounds and α-tocopherol (vitamin E) (Sigma–Aldrich) for the lipophilic products. Solutions of each compouds were prepared at 1.25 mg/ml. Dilutions at 313 and 79 μg/ml were made. Negative controls were the solvents used meaning distilled water for the hydrophilic compounds and ethanol for the lipophilics.

2.5.3. Microplate preparation

Twenty-five ml of samples (1.25 mg/ml), positive and negative controls were added to a microplate (96 wells). The reaction mixture containing 4 ml of liposomes solution, 2.25 ml of phosphate buffer (20 mM, pH 7,4) and 0.25 ml of ascorbate solution (3.1 mg/ml) was prepared. Sixty-five μl of reaction mixture was added to a microplate using a multichannel pipette. Finally, 10 μl of $FeCl_3$ (Sigma–Aldrich) solution (4.3 mg/ml) were added to the wells. The microplate was then incubated at 37 °C for 15 min. One hundred fifty ml of a fresh solution of 10% (v/v) SDS (Sigma–Aldrich) and 0.67% (v/v) thiobarbituric acid (Sigma–Aldrich) in a 1:2 ratio was added in the microplate. The colorimetric reaction was produced at 80 °C for 30 min. The TBARS of the controls and samples were evaluated at 540 nm with a Microplate Autoreader (model EL 309, Biotek Instruments, Winooski, VT). The reaction was calibrated using the positive control whose the antioxidant activity was 100%. The antioxidant activity (AA) was calculated using the following equation:

$$\mathrm{AA}\ (\%) = [\mathrm{OD}_{(\text{negative control})} - \mathrm{OD}_{(\text{sample})}/\mathrm{OD}_{(\text{negative control})} - \mathrm{OD}_{(\text{positive control})}] \times 100.$$

2.6. Determination of Antiradical activity

Free radical scavenging capacities of phenolic compounds from maple sap and syrup collected at different period of the season were evaluated following a procedure of DPD (*N*,*N*-diethyl-*p*-phenylenediamine) colorimetric method as described by others (Le Tien, Vachon, Mateescu, & Lacroix, 2001; Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004). Two hundred ml of sample from a methanolic extract (1.25 mg/ml) were added in a cell containing 3 ml of 0.15 M NaCl and submitted to electrolysis for 1 min (continuous current, 400 V, 10 mA) using a power supply (Bio-Rad, model 1000/500, Mississauga, ON, Canada). After electrolysis, an aliquot of 200 μl was added to 2 ml of DPD solution (25 mg/ml). The generated oxidative species (superoxide anion (O_2^-), singlet oxygen ($^1O_2$) and OH radicals) and their by-products (hydrogen peroxide (H_2O_2) and hypochlorite ion (OCl^-)) react instantly with DPD, producing a red coloration that can be measured at 515 nm using a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). The antiradical activity is equivalent to the capacity of phenolic compounds to inhibit the accumulation of oxidative species (able to oxidize DPD) and consequently the red coloration at 515 nm. The reaction was calibrated using the non- electrolyzed NaCl solution (no oxidative species, ascribed to 100% scavenging) and the electrolyzed NaCl solution (0% scavenging, in the absence of any antioxydants). The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging}\ (\%) = 100 - [(\mathrm{OD}_{\text{sample}}/\mathrm{OD}_{\text{control}}) \times 100],$$

where $OD_{control}$ represents the OD of electrolyzed solution in the absence of phenolic extract and OD_{sample}, the OD of electrolyzed solution with phenolic extract. The OD is directly related to the degree of oxidation of DPD reagent by the oxidative species, meaning that a sample able to reduce completely the level of reactive oxidative species will have a 100% scavenging capacity.

2.7. Determination of antimutagenic activity

The antimutagenicity of phenolic compounds from maple sap and syrup collected at different period of the season was investigated against potassium dichromate and quercetin metabolites in presence of S9 fraction using a modified Umu test. The antimutagenicity per se and of the metabolites was conducted on total phenolic compound extracts from maple sap and syrup samples at 0%, 25%, 50%, 75% and 100% of the season. On the other hand, the glycosylated and aglycone phenolic compounds were analyzed for their antimutagenicity only at 0% and 100% of the season since the middle of the season was not significantly different from those two periods. The Umu test used in this experiment detects the induction of the SOS response following treatment of *Salmonella typhimurium* strain TA1535 with test compounds. This strain carries the plasmid pSK1002 in which the umuC′ gene fused inflame to the lacZ′ gene. The SOS-inducing potency of test compounds would therefore be estimated by the measurement of the induction level of umu operon in terms of intracellular β-galactosidase activity. The SOS response appears after DNA damage or interference with DNA replication (Miyazawa, Sakano, Nakamura, & Kosaka, 2001).

2.7.1. Bacterial strain

Salmonella typhimurium strain TA1535/pSK1002 containing the fusion gene umuC′–′lacZ that produces a hybrid protein with β-galactosidase activity and whose expression is controlled by the umu regulatory region (Oda, Nakamura, Oki, Kato, & Shinagawa, 1985) was purchased from DSMZ (Braunschweig, Germany).

2.7.2. Chemicals

Magnesium chloride ($MgCl_2$) and potassium chloride (KCl) were purchased from Fisher Scientific (Nepean, ON, Canada). SDS, mercaptoethanol, Potassium dichromate, quercetin, the enzyme substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG), glucose-6-phosphate and β-nicotinamide adenine (β-NAD) were purchased from Sigma–Aldrich (Oakville, ON, Canada). Sodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4) were purchased from laboratoires Mat (Beauport, QC, Canada). S9 fraction prepared from livers of male Wistar rats pretreated with Aroclor 1254 was purchased from Invitro Technologies (Baltimore, MD).

2.7.3. Activation mixture

The activation mixture containing 1 ml of S9 fraction, 10 ml of sterile phosphate buffer (0.2 M, pH 7.4), 0.4 ml of 0.4 M $MgCl_2$ + 1.65 M KCl sterile solution, 7.7 ml of sterile distillated water, 0.1 ml of 1 M glucose-6-phosphate sterile and 0.8 ml of 1 M sterile β-nicotinamide adenine (β-NAD) was prepared. This solution must be kept at 4 °C and the S9 fraction and NAD are added last.

2.7.4. Umu test

TGA medium containing 1% Bacto tryptone, 0.5% NaCl, 0.2% glucose and 20 μg/ml ampicilin was inoculated with 1 ml of the tester strain *Salmonella typhimurium* TA1535/pSK1002 and was incubated at 37 °C under moderate agitation during 16 h. The culture was then diluted 10 times with TGA medium and incubated for 2 h or until the bacterial density reached OD_{600} of 0.25–0.3 at 37 °C, resulting in log-phase cells. One ml of the log-phase culture was further added to a test tube containing the test mixture or 1 ml of TGA medium for the control. Two hundred and eight μl of phosphate buffer (0.1 M) for the antimutagenicity per se or 208 μl of the S9 mixture was added for the antimutagenicity evaluation of the phenolic compound metabolites. Forty-two μl of the test phenolic compound solution (1.25 mg/ml) or the solvent (methanol 10% (v/v) for the glycosylated and 100% (v/v) for the aglycone) in which was dissolved the compound (control) was added to each tube. Finally, 42 μl of potassium dichromate (84 μg/ml) or quercetin (313 μg/ml) in presence of the S9 fraction was added to the test tube. The metabolite compounds of quercetin in presence of S9 fraction or the potassium dichromate per se are mutagenic and are used as control. The test mixture and control in test tubes were incubated for 2 h at 37 °C under moderate agitation. At the end of incubation, the cell density from each tube was measured at 600 nm using a spectrophotometer (Unicam model UV4, Cambridge, UK). The β-galactosidase activity was also assayed according to others (Whong, Wen, Stewart, & Ong, 1986). In order to evaluate the β-galactosidase activity, 50 μl of treated cells and 100 μl of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) solution (4 mg/ml in 0.1 M phosphate buffer, pH 7.0) were added to a test tube containing 450 μl of B buffer prepared according to others (Whong et al., 1986) with 16.1 g of Na_2HPO_4, 5.5 g of NaH_2PO_4, 0.75 g of KCl, 0.25 g of $MgSO_4$–7 H_2O, 1 g of SDS, 2.7 ml of β-mercaptoethanol and 1 l of distilled water at pH 7. Finally, the tubes were incubated at 28 °C during 25 min. The enzymatic reaction was stopped by adding 400 μl of 1 M sodium carbonate (Na_2CO_3). The $OD_{420\ nm}$ and $OD_{550\ nm}$ were determined with a spectrophotometer (Unicam, UV4 model, Cambridge, UK). Inhibition of SOS response or antimutagenicity was calculated as follows:

Antimutagenicity (%)
= (β-gal Unit Control-β-gal Unit sample/unit β-gal Control) × 100

β-galactosidase activity was presented as units according to the following formula: Unit = $1000 \times (OD_{420\,nm} - 1.75\ OD_{550\,nm})/(T \times V \times OD_{600\,nm})$ where T represented the time of reaction (min) and V the volume of cells (ml). Enzyme units with a one time dose response were considered as positive results (Miller, 1972).

2.8. Statistical analysis

This experiment was done in replicate and three samples of each replicate were analyzed. Data were analyzed using SPSS for Windows. Analyses of variance were performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range test ($p \leqslant 0.05$).

3. Results and discussion

3.1. Antioxidant and antiradical properties

3.1.1. Total phenolic extracts

The capacity of lipid peroxidation inhibition in relation to the total phenolic content of each extract during the season is illustrated in Fig. 2. The antioxidant properties of phenolic compounds present in maple sap showed antioxidant properties from 84.86% to 96.67% from the beginning of the season to 25% of the season (Fig. 2a). Then a decrease from 96.67% to 42.57% in their antioxidant activity was observed. A maximum of antioxidant activity was observed at the quarter of the season. The concentration of phenolic compounds in the extracts showed an gallic acid equivalent quantity of 16.51–8.51 g/100 g from the beginning of the season to 75% of the season. A value of 24.69 g GEA/100 g was observed at the end of the season. The antioxidant properties of phenolic compounds extracted from maple syrup showed a value of 83.78% (Fig. 2b). Optimal antioxidant properties were observed at 50% of the season representing a value of 95.58% and then a significant ($p \leqslant 0.05$) decrease was observed at 75% of the season and a stable value of 76.02% was observed until the end of the season. The content of the phenolic compounds showed a value of 63.81 g GEA/100 g at the beginning of the season. A significant decrease ($p \leqslant 0.05$) to a value of 17.81 g GEA/100 g was observed until 75% of the season. At the end of the season, a significant increase ($p \leqslant 0.05$) occurred and a value of 59.41 g GEA/100 g was observed. These results showed that there was no correlation ($r^2 < 0.300$) between the

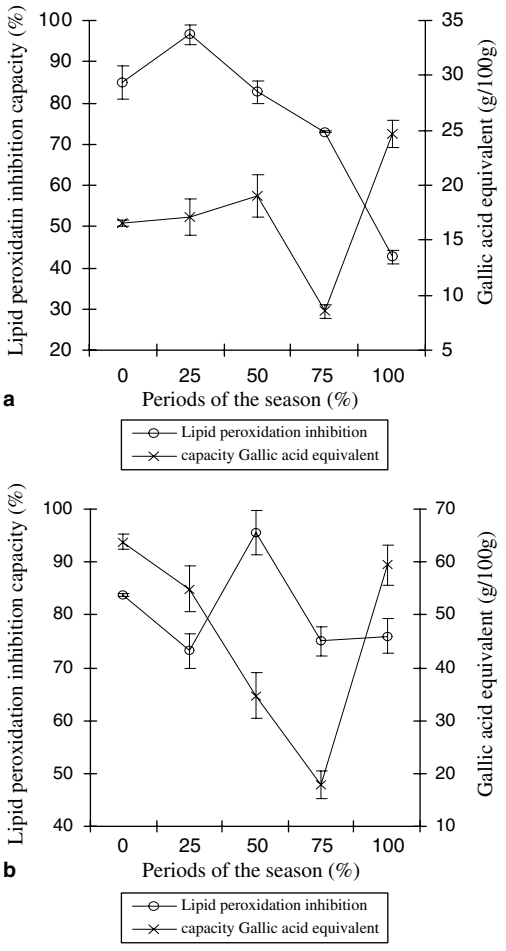

Fig. 2. Lipid peroxidation inhibition capacity and the content of total phenolic compounds in maple sap (a) and maple syrup (b) during the season.

antioxidant activity of maple sap and syrup extracts and the concentration of total phenolic compounds.

The free radical scavenging activity and the content of total phenolic compounds of each extract during the season are presented in Fig. 3. The results showed that the free radical scavenging activity of maple sap extract varies proportionally ($r^2 = 0.947$) with the total phenolic content (Fig. 3a). At the beginning of the season, the extract had a free radical scavenging capacity of 82.95% and it increased at 97.75% at the quarter of the season to remain stable until the end of the mid-season. Then at the three quarter of the season, a significant decrease ($p \leqslant 0.05$) occurred with a free radical scavenging capacity of 67.09%. Finally, at the end of the season, the free radical scavenging capacity increased significantly ($p \leqslant 0.05$) at a value of 75.24%. The free radical scavenging capacity profile of maple sap was different from the one of maple syrup (Fig. 3b). A proportional relation ($r^2 = 0.859$) with the concentration of total phenolic compounds was also observed. At the beginning of the season, the extract had a free radical scavenging capacity of 84.93%. A decrease at

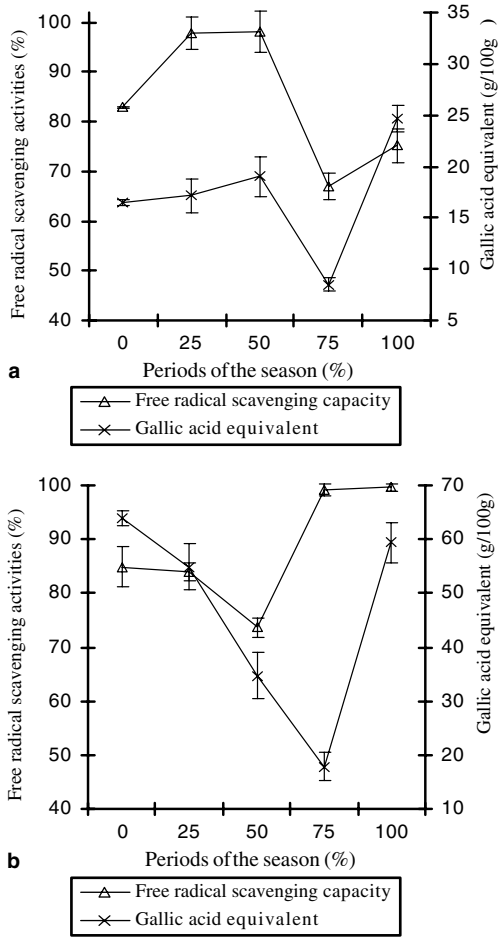

Fig. 3. Free radical scavenging capacities and the content of total phenolic compounds in maple sap (a) and maple syrup (b) during the season.

mid- season (73.62%) and increase were observed at the end of the season (99.63%).

3.1.2. Glycosylated and aglycone compounds

The antioxidant and antiradical properties of the glycosylated and aglycones compounds in maple products extracts was analyzed. The capacity of the glycosylated and aglycone compounds extracted from maple sap to inhibit lipid peroxidation is represented in Table 1. Antioxidant activity values of maple sap extract were found to be superior to 100% regardless of the time of the season, meaning a higher antioxidant activity of the extract than the control. An optimal antioxidant capacity was observed respectively at 25% and 75% of the season with values of 122.46% and 120.63%. A significant decrease ($p \leqslant 0.05$) occurred at the end of the season with a value of 103.69%. The glycosylated compounds from maple syrup, showed also a value superior to 100% until 50% of the season with respective values of 107.89% and 123.14% at 0% and 25% of the season. From 50% to the end of the season, a constant significant decrease ($p \leqslant 0.05$) was observed and a value of the antioxidant

Table 1
Lipid peroxidation inhibition capacity of the glycosylated and aglycone phenolic compounds from maple sap and syrup extracts during the season

Periods of the season (%)	Lipid peroxidation inhibition capacity[a] (%)	
	Sap	Syrup
Glycosylated compounds		
0	113.24 ± 2.66 b	107.89 ± 2.85c
25	122.46 ± 2.03d	123.14 ± 1.93d
50	116.86 ± 2.46bc	100.94 ± 5.31c
75	120.63 ± 2.87cd	70.25 ± 4.09b
100	103.69 ± 1.02a	16.89 ± 4.12a
Aglycone compounds		
0	67.48 ± 3.65b	99.35 ± 1.52b
25	82.73 ± 0.32c	95.41 ± 0.44b
50	96.78 ± 1.45d	127.44 ± 2.67c
75	40.13 ± 0.71a	164.51 ± 3.43d
100	84.67 ± 4.09c	89.75 ± 1.70ab

[a] Means within a column for each type of compound that have different letters are significantly different ($p \leqslant 0.05$). The reaction was calibrated using the ascorbic acid or α-tocopherol (control) whose the lipid peroxidation inhibition capacity was 100%. A lipid peroxidation inhibition capacity value superior to 100% means that maple extract was more antioxidant than control.

activity of 70.25% and 16.89% was respectively observed at 75% and 100% of the season. The results showed that the aglycone compounds showed weaker antioxidant activities. A significant increase ($p \leqslant 0.05$) of the lipid peroxidation inhibition capacity occurred from the beginning to the mid-season. For the aglycone compounds from the sap, a respective value of 67.48%, 82.73% and 96.78% was reached at 0%, 25% and 50% of the season. A significant decrease ($p \leqslant 0.05$) happened at 75% of the season giving a weak value of 40.13% to finally increase at 84.67% at the end of the season. The lipid peroxidation inhibition seems better for the aglycone compounds from the syrup than from the sap. Indeed, the value is similar and respectively of 99.35% and 95.41% at 0% and 25% of the season. The results were superior to 100% at 50% and 75% of the season (127.44% and 164.51%) and decreased significantly ($p \leqslant 0.05$) at the end of the season to a value of 89.75%.

The results of the free radical scavenging activity for the glycosylated and aglycone compounds extracted from maple sap and syrup are presented in Table 2. There is no significant difference ($p > 0.05$) for glycosylated extract present in maple sap at 0% and 50% of the season and the free radical scavenging capacity was, respectively, of 95.42% and 97.75%. At 75% of the season, a significant decrease ($p \leqslant 0.05$) occurred and a value of 69.50% was observed. At the end of the season, a significant increase ($p \leqslant 0.05$) allowed to reach a value of 87.78%. The antiradical properties of the glycosylated compounds from maple syrup extracts was superior to

Table 2
Free radical scavenging capacity of the glycosylated and aglycone phenolic compounds from maple sap and syrup extracts during the season

Periods of the season (%)	Free radical scavenging capacity[a] (%)	
	Sap	Syrup
Glycosylated compounds		
0	95.42 ± 3.59d	94.77 ± 0.4b
25	77.98 ± 1.97b	96.84 ± 0.5c
50	97.75 ± 0.82d	94.72 ± 0.3b
75	69.50 ± 1.13a	90.51 ± 0.61a
100	87.78 ± 1.71c	98.05 ± 2.81d
Aglycone compounds		
0	48.19 ± 1.58b	39.86 ± 1.54a
25	48.28 ± 6.60b	47.24 ± 0.39b
50	89.09 ± 7.58c	79.57 ± 2.94d
75	46.33 ± 0.55b	42.44 ± 3.13ab
100	33.21 ± 4.22a	61.32 ± 2.29c

[a] Means within a column for each type of compound that have different letters are significantly different ($p \leqslant 0.05$).

90% regardless of the period evaluated. At 0%, 25% and 50% of the season, the free radical scavenging acti- vity was 94.77%, 96.84% and 94.72%, respectively. The best antiradical capacity was reached at the end of the season with a value of 98.05%. The aglycone compounds from maple sap showed a weak antiradical activity (inferior to 50%) excepted at mid-season where a significant higher value ($p \leqslant 0.05$) of 89.09% was observed. The aglycone compounds present in maple syrup offered a significant increase ($p \leqslant 0.05$) until mid-season with a value of 39.86%, 47.24% and 79.57%, respectively. A significant decreased ($p \leqslant 0.05$) free radical scavenging capacity occurred at 75% of the season with a weak value of 42.44%. Finally, an increase at the end of the season showed a free radical scavenging capacity of 61.32%.

The results of antioxidant activity of the total phenolic compounds present in the extracts showed some differences. In general, the literature reports that there is a relation between the content of phenolic compounds and the antioxidant property. However, these results showed that other factors should be considered. The nature and the structure of the compounds is indeed very important (Heim et al., 2002). The antioxidant activity of each compound is also important. However a combination of different phenolic compounds structures was observed in maple extracts and some compounds are more active than others. Furthermore, as reported by Shahidi (2000), the efficacy of natural antioxidant contained in bulk oils, emulsions and composite food might be greater than that of individual phenolic compounds. The technological process to manufacture maple syrup can also influence the antioxidant activity since it influences the phenolic content (Kermasha et al., 1995). In addition to the phenolic compounds, Maillard reaction products (MRPs) were found in maple syrup (Akochi, Alli, Kermasha, Yaylayan, & Dumont, 1994). Since these compounds could have been extracted in the organic phase with the phenolic compounds they can add to the antioxidant capacity of the extracts. Indeed, it has been reported that some MRPs found in food processed by heat treatments showed antioxidant properties (Anese, Manzocco, Nicoli, & Lerici, 1999).

The antiradical activities varies also according to the nature of the compounds. Indeed, the glycosylated moety influence the antioxidant properties (Heim et al., 2002). In general, the glycosylated compounds have a weaker antiradical activity than their aglycone equivalent (Rice-Evans, Miller, & Paganga, 1996). However, the present study showed that the glycoside compounds of the maple sap and syrup phenolic extracts had a better antiradical activity than the aglycone compounds. The spatial arrangement of substituents is a greater determinant of antiradical activity than the flavan backbone (Rice-Evans et al., 1996). Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antiradical activity (Burda & Oleszek, 2001; Cao, Sofic, & Prior, 1997). Free radical scavenging capacity is primarily attributed to the high reactivities of hydroxyl substituents. The B-ring hydroxyl configuration is the most significant determinant of scavenging of reactive oxygen species (Burda & Oleszek, 2001). Hydroxyl groups on the B-ring donate hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. Among structurally homologous flavones and flavanones, peroxyl and hydroxyl scavenging increases linearly and curvilinearly, respectively, according to the total number of OH groups (Cao et al., 1997). The differences in antiradical activity between polyhydroxylated and polymethoxylated flavonoids are most likely due to differences in both hydrophobicity and molecular planarity (Heim et al., 2002).

3.2. Antimutagenicity properties

3.2.1. Total phenolic extracts

Results of the antimutagen properties per se of total phenolic compounds extracted from maple sap and syrup are shown in Table 3. The β-galactosidase units, represent the specific enzymatic activity of the β-galactosidase produced by *Salmonella typhimurium* TA1535/pSK1002 due to induction of the SOS response in presence of mutagen agents (McDaniels, Reyes, Wymer, Rankin, & Stelma, 1990). The SOS-inducing potency of test compounds is estimated by the measurement of the induction of the level of umu operon in terms of intracellular β-galactosidase activity (Ong, Stewart, Wen, & Whong, 1987). The percentage of antimutagenicity is calculated to illustrate the inhibition of the SOS response. The results of the β-galactosidase activity at

Table 3
Antimutagenicity per se of total phenol extracts from maple sap and syrup during the season

Samples[a] (%)	β-Galactosidase units[c]		% Antimutagenicity[d]	
	Sap	Syrup	Sap	Syrup
Control[b]	442.31 ± 17.69c	402.31 ± 25.90a	N/A	N/A
0	426.79 ± 12.69bc	396.02 ± 9.51a	3.51	1.56
25	436.68 ± 16.98bc	405.31 ± 26.46a	1.27	−0.75
50	408.24 ± 6.79ab	488.63 ± 27.01b	7.70	−21.45
75	508.98 ± 26.28d	524.43 ± 14.11b	−15.07	−30.35
100	387.25 ± 8.24a	506.74 ± 11.13b	12.45	−25.96

[a] Samples are phenolic extracts collected during the season 0 being the beginning and 100% the end of the season.
[b] Control is the mixture containing only the mutagen agent and the cells.
[c] β-Galactosidase units represents the enzymatic specific activity. Means in the same column bearing the same letter are not significantly different ($P > 0.05$).
[d] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.

0% and 25% of the season are, respectively, 426.79 and 436.68 units for the maple sap extracts and no significant difference ($p > 0.05$) was observed between the control and those samples. At mid-season, a significant decrease occurred ($p \leqslant 0.05$) giving a value of 408.24 units, followed by an increase at 75% of the season to reach 508.98 units. However, a significant decrease ($p \leqslant 0.05$) to a value of 387.35 units was observed at the end of the season. The values of β-galactosidase activity of phenolic extracts from maple syrup showed that at the beginning of the season (0% and 25%) the samples are not significantly different ($p > 0.05$) than the control with respective values of 396.02 and 405.31 units. At mid-season until the end of the season, the values of β-galcatosidase activity increased significantly ($p \leqslant 0.05$) to values of 488.63, 524.43 and 506.74 units at 50%, 75% and 100% of the season, respectively. The percen- tage of antimutagenicity calculated in maple sap was 3.5% at the beginning of the season. At 50% of the season, there is a slight but significant increase ($p \leqslant 0.05$) representing a value of 7.7%. Then at 75% of the season, there is a significant decrease ($p \leqslant 0.05$) of the antimutagenic activity and the obtained value is negative (−15.07%). Finally, there is a significant increase ($p \leqslant 0.05$) of the antimutagenic activity at the end of the season to reach 12.45% of antimutagenicity. The values of percentage of antimutagenicity showed that there is no antimutagenic activity per se for the maple syrup total phenol extracts.

The results for the antimutagenic properties of the metabolites of total phenolic extracts from maple sap and syrup are presented in Table 4. The data of β-galactosidase units obtained for the maple sap showed that only the values obtained for the samples at 0 and 100% of the season are significantly ($p \leqslant 0.05$) different from the control with respective values of 492.56 and 484.45 units compared to 571.16 units for the control. The values at 25%, 50% and 75% of the season were respectively of 569.77, 556.49 and 520.04 units. No significant difference was observed between these values when compared with the control. There is no significant difference ($p > 0.05$) among the values at 75% and 100% of the season. The β-galactosidase activity of the metabolites of maple syrup extracts confirms that there is no significant difference ($p > 0.05$) between samples at 0%, 25%, 50% and 75% of the season and the control. These values are respectively 625.22, 597.03, 614.11 and 652.99 units compared to 621.47 units for the control. A significant decrease ($p \leqslant 0.05$) is visible at 100% of the season where a value of 529.12 units is observed. The antimutagenic activity of the metabolites of the maple sap extracts indicated that at the beginning of the season a value of 13.76% is observed. Then, a significant decrease ($p \leqslant 0.05$) at the quarter of the season occurred to a value of 0.24%. At 50% and 75% of the season the values obtained are respectively of 2.57% and 8.95%. A significant increase at the end of the season allowed reaching a maximum of 15.18%. The total phenolic extracts metabolites from maple syrup showed a weak percentage of antimutagenicity. The percentages of antimutagenicity of the metabolites of phenolic compounds extracted in syrup, were, respectively, of −0.6%, 3.93%, 1.18% and −5.07%, at 0–75% of the season. At the end of the season an improvement was observed to reach a value of 14.86%.

The antimutagenic activity of the metabolites of total phenolic extracts observed showed a low antimutagenic potential but the value obtained was higher than those obtained for the antimutagenicity per se of the same compounds. This means that the compounds are getting their antimutagenic activity after being metabolized in the liver in presence of S9 fraction. The phenolic compounds, especially the flavonoids, possess antioxidant properties, but they are not necessarily able to prevent all kind of mutations that can be induced by this mutagen agent. It has been reported that the phenolic compounds can serve as screens against UV radiation in plants (Escarpa & Gonzalez, 2001). Hence, they can be efficient in preventing mutations in plants but they might need to be metabolized to produce an antimutagenic activity in another organism.

Table 4
Antimutagenicity of total phenolic extracts metabolites from maple sap and syrup during the season

Samples[a] (%)	β-Galactosidase units[c]		% Antimutagenicity[d]	
	Sap	Syrup	Sap	Syrup
Control[b]	571.16 ± 22.93b	621.47 ± 39.70b	N/A	N/A
0	492.56 ± 12.00a	625.22 ± 35.98b	13.76	−0.60
25	569.77 ± 23.92b	597.03 ± 1.65b	0.24	3.93
50	556.49 ± 33.35b	614.11 ± 18.78b	2.57	1.18
75	520.04 ± 33.06ab	652.99 ± 42.52b	8.95	−5.07
100	484.45 ± 24.08a	529.12 ± 40.91a	15.18	14.86

[a] Samples are phenolic extracts collected during the season 0% being the beginning and 100% the end of the season.
[b] Control is the mixture containing only the mutagen agent and the cells.
[c] β-Galactosidase units represents the enzymatic specific activity. Means in the same column bearing the same letter are not significantly different ($P > 0.05$).
[d] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.

Also, the mutagen agent used for the investigation of the antimutagenicity per se is the potassium dichromate, a strong oxidant. The mutations caused by the potassium dichromate can differ in nature from the damage inflicted by UV radiation and then, escape the protection or repair mechanism of bacteria. Furthermore, the phenolic compound extracts were complex mixtures, meaning that different phenomenons such as synergy or co inhibition can interfere with the antimutagenic activity. Kaur and Saini (2000) have associated antimutagenicity with antioxidant properties, due to the capacity of the compounds to inhibit the DNA damage caused by the presence of free radicals. In fact, inhibition of mutagenesis is generally not based on one specific mechanism. Compounds and complex mixtures with antimutagenic activity have different modes of action and act in parallel at different levels. As inhibitors, they may prevent the formation of mutagens. According to others (Krul et al., 2001), as blocking agents, they can prevent the biotransformation of premutagens into reactive metabolites by inhibiting metabolic activation or by scavenging reactive molecules. As suppressing agents they may modulate intracellular processes, which are involved in DNA repair mechanisms.

3.2.2. Glycosylated and aglycone compounds

The antimutagenic properties per se of glycosylated and aglycone phenolic compounds at the beginning and the end of the season are shown in Table 5. The percentages of antimutagenicity show that both phenolic extracts from sap and syrup are inferiors to zero and hence do not present any antimutagenic activity. The results obtained in sap showed a mean value of −15% at the beginning of the season and −13% at the end of the season for both extracts. In the syrup, a value of −56.65% and −31.25% was observed at the beginning of the season for the glycosylated and aglycone extracts, respectively. At the end of the season, the valued obtained for the same compounds were, respectively, −40.46% and −13.63%. Those results could indicate a mutagenic potential, but according to Whong et al. (1986) to be considered a mutagenic positive result with the Umu test the β-galactosidase unit should be one time or more increased over the control, meaning in this case a percentage of −100% or more antimutagenicity.

Table 5
Antimutagenicity per se of glycosylated and aglycones phenolic compounds extracted from maple sap and syrup at the beginning and the end of the season

Period of the season[a] (%)	% Antimutagenicity[b]			
	Glycosylated		Aglycones	
	Sap	Syrup	Sap	Syrup
0	−15.82	−56.65	−14.75	−31.25
100	−13.24	−40.46	−12.66	−13.63

[a] Samples are phenolic extracts collected at different periods of the season 0 being the beginning and 100% the end of the season.
[b] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.

The antimutagenic activity of the metabolites of glycosylated and aglycone phenolic extracts are illustrated in Table 6. The results showed that the antimutagenic properties of glycosylated compounds in sap were of 3.00% at the beginning of the season and of −18.16% at the end of the season. The values of percentages of

Table 6
Antimutagenicity of glycosylated and aglycone phenolic compounds metabolites extracted from maple sap at the beginning and the end of the season

Period of the season (%)[a]	% Antimutagenicity[b]			
	Glycosylated		Aglycones	
	Sap	Syrup	Sap	Syrup
0	3.00	14.05	−24.81	42.33
100	−18.16	11.81	ND[c]	31.39

[a] Samples are phenolic extracts collected at different periods of the season 0% being the beginning and 100% the end of the season.
[b] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.
[c] Not determined.

antimutagenicity obtained for the glycosylated compounds present in syrup were 14.05% and 11.81% at 0% and 100% of the season respectively. The results for the aglycone compounds present in maple syrup showed the highest values. At the beginning of the season, a percentage of antimutagenicity of 42.33% was obtained. Then at the end of the season, the percentage was 31.39%.

The results obtained for the metabolites of aglycone present in maple syrup showed a higher antimutagenic activity than those from maple sap. These results could be explained by the creation of new chemical compounds produced during the processing of maple sap to syrup. For example, Maillard's reactions produced during the heating can produce new compounds with antimutagenic activity. The antimutagenic activity of glycosylated compound is weak and similar when present in both sap and syrup. These results can be explained by the fact that the glycosylated compounds are more stable and less active than the aglycones. As for the total phenolic extracts there is no antimutagenicity per se for the glycosylated and aglycone compounds from maple sap and syrup.

4. Conclusion

The results observed in this study showed that the phenolic compounds present in maple sap and syrup have antioxidant and antiradical activities. The present work indicated that a variation of the antioxidant activity of the phenolic compounds in maple sap and syrup is observed throughout the season. Also, the nature of the compounds, glycosylated and aglycone, also influenced the lipid peroxidation inhibition and the free radical scavenging capacities. The results obtained in this study have also illustrated the antimutagenic potential of phenolic compounds present in maple sap and syrup, except for the per se glycosylated and aglycone compounds. The aglycone compounds present in sap and syrup are efficient to prevent the mutations in cells. However, the efficienty level in both products is related to the season period.

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